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The effect of MinC on FtsZ polymerization is pH dependent and can be counteracted by ZapA

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2 **Supplemental material :**

3 **- Supplemental methods**

4 **- Supplemental Figure Legends**

5

6 **Supplemental methods.**

7 *Protein purification.* FtsZ was essentially purified as described [22]. FtsZ was
8 precipitated from a membrane-free cell extract with a 30-40% ammonium sulphate
9 cut, resuspended in 50 mM Tris/HCl; 50 mM KCl; 1 mM EDTA; 10% v/v glycerol
10 pH 8.5 and loaded on a MonoQ HR10/10 column (Amersham Biosciences). The
11 column was eluted with a 50-500 mM KCl gradient. Purified FtsZ was dialysed
12 against 50 mM Hepes/NaOH; 1 mM EGTA; 2.5 mM MgAc; 10% v/v glycerol; 50 μ M
13 GDP pH 7.9, frozen in liquid nitrogen and stored at -80°C.

14 MalE and MalE-MinC were purified by amylose column chromatography as
15 described [5].

16 For the purification of MinC-strep and MinC19-strep, overnight cultures of BL21
17 (DE3) carrying pDJ15 or pDJ16 were diluted 1:100 in fresh LB. Cultures were grown
18 to $A_{660} \sim 0.6$, induced by the addition of anhydro-tetracyclin (0.2 μ g/ml) and grown
19 for an additional 3 hours. Cells were harvested (10 min; 7000 x g; 4°C), washed with
20 ice cold TBS (24.8 mM Tris; 2.7 mM KCl; 137 mM NaCl; pH 7.4) and resuspended
21 in 50 mM Hepes/NaOH; 150 mM NaCl; 1 mM EDTA pH 7.4 (Buffer A) with a
22 Complete EDTA-free protease inhibitor tablet (Roche). All subsequent steps were
23 carried out at 4°C or on ice. Cells were disrupted by three passages through a French
24 Press at 8000 psi. Cells, debris, and membranes were removed by two centrifugation
25 steps: 10 min at 8000 x g followed by 60 min at 220,000 x g. Avidin (Sigma) was

1 added to 0.02 mg/ml, 4 ml of Strep-Tactin-Sepharose (IBA technologies GmbH) was
2 added and the suspension was incubated overnight at 4°C with mild mixing. The
3 suspension was loaded on a column and the Sepharose resin was allowed to settle.
4 The column was washed with 12.5 volumes of buffer A and eluted with 3 volumes of
5 buffer A containing 5 mM desthiobiotin. MinC-strep containing fractions were diluted
6 four times with 50 mM Hepes/NaOH; 1 mM EDTA; 10 % v/v glycerol pH 8.1 and
7 loaded onto a MonoQ HR10/10 column (Amersham Biosciences). The column was
8 eluted with a 0-500 mM NaCl gradient with MinC-strep eluting at ~ 50 mM NaCl.
9 Concentrated MinC-strep was frozen in liquid nitrogen and stored at -80°C.

10 For the purification of His₈-ZapA, overnight cultures of BL21 (DE3) carrying pDJ26
11 were diluted 1:100 in fresh LB. Cultures were grown to A₆₆₀ ~ 0.6, induced by the
12 addition of iso-propyl-β,D-thio-galactopyranoside (IPTG) to 1 mM and grown for an
13 additional 2 hours. Cell extracts were prepared as described for MinC-strep, only 50
14 mM Hepes; 500 mM NaCl; 10 mM Imidazole; 1 mM PMSF pH 7.0 was used as the
15 resuspension buffer. Extracts (~10 ml) were incubated with 4 ml of a 50% slurry of
16 Ni-NTA agarose (Qiagen) for one hour with mild mixing. The suspension was loaded
17 on a column and the Ni-NTA agarose resin was allowed to settle. The column was
18 washed with 10 ml of resuspension buffer, 40 ml of resuspension buffer containing 20
19 mM imidazole and eluted with 15 ml of resuspension buffer containing 200 mM
20 imidazole. His₈-ZapA containing fractions were used immediately.